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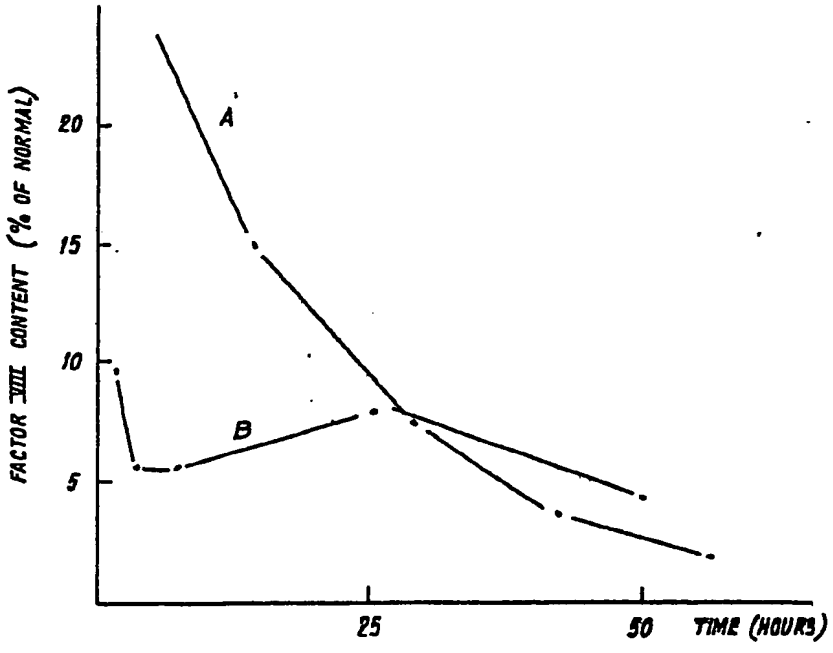

(57) Abstract

Pharmaceutical compositions containing liposomes formed from phospholipids in the presence of blood clotting factor VIII (anti-hemophilic factor) have been found to be active on oral administration by increasing the factor VIII blood plasma level. The invention relates to such pharmaceutical compositions and to methods for the preparation thereof.

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Pharmaceutical composition and process for the preparation thereof.

The present invention relates to a pharmaceutical composition containing antihemophilic factor (factor VIII) as an active substance, as well as to a process for the preparation thereof.

Hemophilia A is a disease in which clotting factor VIII is present as an inactive genetic varation. In Von Willebrand's disease clotting factor VIII is present in the blood plasma in low concentrations. Both of the diseases are characterised by abnormal bleeding tendency. The bleedings may be life-threatening, but may also lead to serious invalidity, among others by hemarthrosis and muscular atrophy.

Therapy consists of administration of factor VIII. This can be effected via the intravenous route only, because factor VIII is very much susceptible to attack by proteolytic enzymes. Therefore, oral administration of factor VIII does not result in uptake thereof in the blood. Administration of factor VIII is used in case of bleedings and, prophylactically, before surgical operations or in situations of increased bleeding risk (for example in practising sports). The disadvantage of this way of administration is the necessity of vena puncture. Although there are patients capable of injecting themselves, the requirements of sterility and of technique of vena puncture are of such a nature that, in most cases, help in a medical centre is needed. Further, the necessary vena puncture is sometimes difficult, such as with obese patients or with patients having cicatricated veins due to repeated puncture. Therefore, oral treatment of hemophilia is highly desirable.

It was found that pharmaceutical compositions containing anti-hemophilic factor (factor VIII) as an active substance are effective on oral administration if

the anti-hemophilic factor is incorporated into liposomes formed from phospholipids.

Liposomes formed from phospholipids are understood to be structures consisting of a number of concentric layers (comparable with those of an onion) the total diameter of which may be 500 - 1000 nm. These liposomes will be formed when phospholipids are suspended in water. In case the liposomes are formed, that is to say the phosphatides are suspended, in a solution of anti-hemophilic factor (factor VIII) this substance is also trapped between the layers. The result is that the substance becomes, as it were, packaged, and that the substance is highly protected in the gastro-intestinal tract, so that the substance cannot be broken down before its introduction into the blood stream.

The carrier material used, that is to say the lipids, are normal body constituents and are also normal constituents of our food (namely of egg, fish and meat) so that they may be considered non-toxic.

The preferred phospholipid from which the liposomes according to the invention are formed is egg-lecithin although, in principle, all lecithins (for example soya lecithin) may be used. Egg lecithin is readily available, however, and does not contain toxic constituents.

Preferably, the liposomes are formed from a mixture of phospholipids with a nett charged lipid. This lipid introduces a charge in the double-layer of the phospholipid, thereby increasing the distance between the double layers. This facilitates the uptake of factor VIII between the layers of the phospholipid. Fatty alcohol phosphates, such as dicetyl phosphate, but also phosphatidic acids and free long chain fatty acids, such as oleic acid, may be mentioned as examples of such charged lipids. Preferably, the charged lipid is a phosphatidic acid, especially a natural phosphatidic acid. Last-mentioned natural phosphadidic acids are generally mixtures in which the fatty acid radicals are derived from palmitic acid,

stearic acid, and oleic acid.

The invention also relates to a process for preparing a pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance. This process is characterised by the fact that phospholipid liposomes are formed in the presence of factor VIII. Generally, this process is carried out by suspending a phospholipid in an aqueous solution of factor VIII. This suspending operation may be effected with the aid of ultrasound but also, quite easily, by dissolving the phospholipid in a volatile solvent, such as ethanol, bringing the solution into a flask and concentrating the solution in the flask in such a way that the phospholipid is deposited as a thin layer on the inner wall of the flask. A milky suspension of liposomes will be formed by adding an aqueous solution of factor VIII and, after addition of some glass beads, swinging vigorously. The liposomes may be removed by centrifugation and, if desired, may be re-suspended in water or in a physiological salt solution. The liposome suspension may be processed further to form a liquid mixture for oral administration which may contain, for example, taste corrigentia. Also, the concentrated or diluted liposome suspension may be brought into soft gelatin capsules. It is also possible to prepare compositions which pass the stomach unchanged and become active only in the intestines.

Plasma fractions enriched with respect to factor VIII may be prepared in various ways. In this connection reference can be made to Vox Sanguinis 30 (1976) pages 1-22. In practice a plasma fraction enriched with respect to factor VIII may be obtained most readily by keeping plasma for some time at a low temperature. This results in the formation of a precipitate which may be removed by centrifugation. The precipitate is called cryoprecipitate and it is excellently suitable for use in the present compositions. Cryoprecipitate contains fibrinogen as well. Surprisingly it was found that a

larger percentage of factor VIII than of fibrinogen is incorporated into the liposomes. Therefore, the pharmaceutical compositions according to the invention contain less fibrinogen per unit of weight of factor VIII than the cryoprecipitate used as the starting material.

In the process according to the invention the preferred phospholipid is egg lecithin, especially egg lecithin mixed with a charged lipid. Preferably the charged lipid is a phosphatidic acid, especially a natural phosphatidic acid.

The advantages of the compositions according to the invention are evident. The method of preparation is simple. Moreover, it is not necessary to carry out the preparation under strictly sterile conditions. It is desirable, however, to take care that the composition cannot deteriorate due to attack by micro-organisms.

Although only about 30% of the amount of factor VIII present in the compositions will be introduced into the blood stream after oral administration, the compositions according to the invention have the advantage of a retarded release of factor VIII into the blood. On intravenous administration which, of course, results in 100% uptake of factor VIII into the blood stream, a half life of 14 hours is observed. The effect of the retarded release results in a blood level during the second day after administration of the present compositions which is equal to or even higher than the level obtained on intravenous administration.

The following example illustrates the invention.

Example.

Preparation of the composition.

To each of two roundbottom flasks of 250 ml are added 50 ml of an egg lecithin solution (Koch-Light; 1 g/10 ml ethanol) and 10 ml of a phosphatidic acid solution (Koch-Light; 20 mg/ml chloroform).

The solvents are removed by evaporation at about 45°C by menas of a rotation evaporator connected to a

waterjet pump. In this way the lipids remain as a thin layer on the inner wall of the flask. To one of the flasks 100 ml of an isotonic (0.9% by weight of NaCl) solution of an enriched factor VIII preparation (AHF-Konzentrat SRK (human); Zentral Laboratorium Blutspendedienst SRK Switzerland; 10 ml of lyophilised preparation contains about 230 U of factor VIII) are added, as well as some glass beads. The mixture is swung vigorously by hand until the lipid film is removed from the wall (5 to 10 minutes). The milky suspension obtained is centrifuged at 27.000 x g during 20 minutes which results in floating of the liposomes. The lower liquid layer is added to another flask and is shaken again in the presence of glass beads until the formation of the liposomes is complete. After centrifugation, the amount of non-trapped factor VIII present in the lower liquid layer, may be determined. The liposome fractions of both of the centrifugations are mixed. The mixture is suitable for oral administration as such or after dilution with physiological salt solution and/or addition of taste corrigentia.

In this process the amount of factor VIII entrapped was about 80%. The amount of fibrinogen entrapped was only 25% which means an enrichment with respect to factor VIII.

Factor VIII was essayed with the "one stage" method of Veldkamp (Thrombos. Diathes haemorrh. 19, 279 (1968)).

Results.

A typical experiment is illustrated in the attached drawing. At time 0 (fasting patient) 800 units of factor VIII were administered per os in the form of the composition obtained as described above. This results in an increase of the factor VIII level in the plasma to about 10% of the normal value. The plasma level remains above about 5% of the normal value during 50 hours. It is remarked in this connection that a factor VIII level of $> 5\%$ is regarded to be a status of light hemophilia, that is to say, a clinical picture in which spontaneous bleedings occur very rarely only. Thus, the low dosis

according to the example provides already an effective prophylaxis during 48 hours.

In the drawing curve A indicates the change of concentration in case the same amount of factor VIII would have been administered intravenously. Curve B indicates the change as observed after oral administration of the composition according to the invention. It should be noticed that the patient suffered from hematuria before the administration, and that this phenomenon was absent from 2 to 6 hours after the administration, and returned after that time. Oral administration of factor VIII as such and of phospholipid as such had no effect whatsoever on the factor VIII level.

C L A I M S.

1. Pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance, characterised in that the composition is suitable for oral administration and that factor VIII is incorporated in liposomes formed from phospholipids.

2. Composition according to claim 1, characterised in that the liposomes have been formed from phospholipids and a nett charged lipid.

3. Composition according to claim 1 or 2, characterised in that the phospholipid is egg lecithin.

4. Composition according to claims 1 - 3, characterised in that the nett charged lipid is a phosphatidic acid.

5. Composition according to claim 4, characterised in that the phosphatidic acid is a natural phosphatidic acid having oleic acid, palmitic acid and stearic acid radicals as fatty acid constituents.

6. A process for preparing a pharmaceutical composition containing anti-hemophilic factor (factor VIII) as an active substance, characterised by forming phospholipid liposomes in the presence of factor VIII.

7. The process of claim 6 characterised by suspending a phospholipid in an aqueous solution of factor VIII.

8. The process of claim 6 or 7, characterised by suspending a phospholipid and a nett charged lipophilic substance in an aqueous solution of factor VIII.

9. The process of claims 6 - 8, characterised in that the phospholipid is egg lecithin.

10. The process of claims 6 - 8, characterised in that the nett charged lipophilic substance is a phosphatidic acid.

11. The process of claim 10, characterised in that the phosphatidic acid is a natural phosphatidic acid containing oleic acid, palmitic acid and stearic acid radicals as fatty acid constituents.

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